

TRIALS AND ABSTRACTS

**MANIPULATION OF INTESTINAL MICROFLORA WITH LIVE
BACTERIA ON THE CLINICAL COURSE OF DIARRHOEA IN
CHILDREN**

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ABSTRACT

Infection of pathogenic bacterial in diarrhoea will result in changes of intestinal micro ecology and the defence mechanism of intestinal microbe colonization. In order to overcome the disadvantaging process, reversal of intestinal flora integrity back to normal is necessary by making an improvement to the normal defence of microorganism colonization. Imbalance of intestinal microflora leads to the occurrence of diarrhoea. Manipulation, by combining probiotics and prebiotics (synbiotic), is able to improve the clinical course of acute diarrhoea.

Objective: To manipulate the clinical course of acute diarrhoea in children (the remission rate and duration of remission) with the administration of synbiotics.

Methodology: Children aged between 6 months and 2 years suffering from acute diarrhoea, were allocated for study using a randomised double blind placebo control clinical trial experimental design. The patients were randomly assigned into a synbiotic group and a control group.

Result and Discussion: 25 children in the synbiotic group and 25 in the control group. No differences in subject characteristic and confounding variables were found in either group. In the synbiotic group, a more rapid improvement in the remission rate was significant compared to the control group ($p = 0.002$). Similar results were also shown in the analysis of faecal form and frequency of daily faecal excretion where there was a significant decrease in the synbiotic group. The average duration of remission in the synbiotic group was 37 hours; this was significantly different when compared to the control group average of 72 hours

Conclusion: The administration of the synbiotic in acute diarrhoeal patients can rapidly improve the remission rate and duration of remission in order to shorten and prevent any further transmission of pathogen.

Introduction

Background of the case

Diarrhoea is still the main health problem in Indonesia. Antibiotics are often used without any clear indication and may cause an imbalance of the intestinal flora. Pathogenic bacteria infection itself may cause intestine microecology and intestine microbe colonization defence. The changes of normal intestine microflora in human may cause the disturbances to the intestine which would emerge as a disease. To solve this problem the returning of normal intestinal flora integrity by fix colonization defence of normal microorganism is needed. Probiotics could accelerate the remission process of acute diarrhoea but is not yet optimal without any prebiotic increment. Colonization by probiotics to form the normal microecosystem could be manipulated by diet adjustment which contains both combinations (synbiotics). Prebiotics cannot be separated with probiotics because the prebiotic target is to accelerate the selective growth of probiotic bacteria. Prebiotics increase the live defence rate of probiotic bacteria because of the specific substrate which has been provided for the fermentation process so the body will get more benefit from this combination. The objective of this research is to regulate the changes of microecosystem in acute diarrhoea in children (remission rate and remission duration) by giving synbiotics.

RESEARCH METHOD

Material and principal work

This research used a randomised double blind placebo control clinical trial experimental design. This research was held in Gastroenterology division department of the Child Health Dr. Soetomo Hospital in February and March 2004. The population in this research are acute diarrhoea patients who were cared for in the Gastroenterology Division Department of the Child Health Dr. Soetomo Hospital, while the sample included all of the acute diarrhoeal patients and willing to participate of this research. Inclusion criteria included: children aged 6 to 24 months, with a diarrhoea history, in this case diarrhoea more than three times with liquid consistency. While the exclusion criteria included diarrhoea that had been passed more than three days in their home, severe malnutrition, or who had been treated with probiotic formula, or who had been found to have another disease which could be affecting the course of diarrhoea.

Acute diarrhoea is defined as stool excretion with liquid consistency that has occurred more than three times in a day and has run for less than 7 days. Diarrhoea remission rate in this research is divided into: Grade I remission if the frequency stool excretion is not more than three times in a day within solid formed consistency, Grade II remission if the stool excretion is not more than three times in a day with sticky consistency. Grade III remission if the frequency of stool excretion is not more than three times in a day, with liquid consistency with residue. Acute diarrhoea is classed as not in remission if the frequency of the stool excretion still more than three times in a day with liquid consistency and has gone

beyond the 7th day of diarrhoea and has been given standard treatment. Remission time is the period of time (in hours) since admission to hospital until reaching a Grade I remission state. The changes of enteropathogen are the result of stool culture at the beginning of the research compared with the stool culture at the end of research. The synbiotic formula is a capsule which contains lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophilus 2×10^8 cfu/gram and the prebiotic Fructooligosaccharide (FOS).

Patients, who met the inclusion criteria were given a physical examine and then randomly selected for treatment groups. The patient treatment group was given synbiotics once in a day and the control group patients were given a placebo with same dosage and method. All of the patients were evaluated every day in stool excretion and the texture of the stool so it could be determined the diarrhoea remission rate (remission grade I, II, III). This evaluation was done until the patient could be discharged from the hospital. If the diarrhoea was still infected after more than 7 days, it was stated that the acute diarrhoea was not in remission and the patient was still evaluated until the frequency of stool excretion and the texture of the stool met the criteria of rate I remission. If the patient removed themselves from this research or was forced to go home before completing the remission process, then they were deemed a failed test subject.

RESULT OF THE RESEARCH

Samples of this research were gathered during February and March 2004. During this period 50 patients were found with acute diarrhoea, 25 patients were placed in the synbiotic group and 25 patients in the control group.

Research Subject Characteristics

No significant differences were found between the synbiotic group and the control group (table 1)

Stool culture result.

Examination results showed that aerobic bacteria, 34% has been identified as *E coli enteropathogenic* (28%), beside *Klebsiella pneumoniae* (4%), *Klebsiella oxytoca* (2%), Anaerobe bacteria (probiotic) examination was found positive culture to the 44% patients. Rotavirus examination has found positive result in 36% patients. No significant differences were found in the stool culture (anaerobe bacteria and rotavirus) in the acute diarrhoeal patients between the synbiotic group and the control group ($p < 0.05$), except the aerobe bacteria.

Table 1. Subject Characteristics

	Probiotic group n = 25	Control group n = 25	P
Age (months, mean $\pm$ SD)	10.6 $\pm$ 4.5	11.56 $\pm$ 5.4	0.498 (NS)
Sex (% male)	31	19	0.561 (NS)
Nutrition status (%)			
- Moderate malnutrition	5	7	0.635 (NS)
- Good	20	18	0.742 (NS)
Breastfeeding (%)	13	19	0.140 (NS)
Diarrhoea onset (days, mean $\pm$ SD)	2.4 $\pm$ 0.8	2.36 $\pm$ 0.8	0.722 (NS)
Stool excretion frequency (x/day, mean $\pm$ SD)	8.9 $\pm$ 2.9	9.6 $\pm$ 4.4	0.217 (NS)
Stool texture (%)			
-Liquid without residue	3	3	1.000 (NS)
-Liquid with residue	12	12	
Sign and symptom (%)			
- Vomiting	18	17	1 (NS)
- Cough and cold	16	15	1 (NS)
- Fever	23	22	1 (NS)
- Dyspnea	1	1	1 (NS)
The last 3month diarrhoea (%)			
- Never	12	15	0.390 (NS)
- Once	7	5	
- 2 times	2	4	
- 3 times >	3	1	
Dehydration rate (%)			
- Without dehydration	1	1	0.761 (NS)
- Mild dehydration	6	5	
- Moderate dehydration	18	19	
- Severe dehydration	-	-	
	12	13	1 (NS)

Positive urine culture at the beginning of study (%)			
Stool malabsorption test (%)	3	6	0.463 (NS)
- Clinic test (+)	21	19	0.725 (NS)
- Floating test (+)			
Positive stool at the beginning (%)			
- Anaerobe bacteria	11	6	0.232 (NS)
- Rotavirus	3	11	0.773 (NS)
- E.coli	10	4	NA

S=significant, NS=Non significant, NA=Non Analysis

Diarrhoea remission rate

Based on analysis of the stool texture (table 2) and stool excretion frequency for each day (table 3) during the examination, an improvement to the stool texture and the decreasing of stool excretion frequency in each day between synbiotic group and control group which has a statistically significant difference ($p < 0.05$) was found.

For the first day of examination there were 18 patients who were stated as at rate II remission in the synbiotic group and 16 patients in the control group. It was found 7(28%) of patients were rate III remission and 9(49%) patients rate III remission and 9 patients were rate III remission in synbiotic group.

For the second day examination, 14 (56%) patients were stated as having rate I remission and 11 (44%) patients rate II remission in the synbiotic group. In the control group 1 (4%) patients was at rate I and 23 (92%) were at rate II remission. Significant difference was found in diarrhoea remission rate at the second day examination between synbiotic group and the control group ($p < 0.05$).

At the third day examination 25 (100%) patients were stated as rate I remission in the synbiotic group, while in the control group it was found that 16 (64%) patients were rate I remission and 9 (36%) patients were in rate II remission. Significant differences were found in the remission rate on the third day between the synbiotic group and the control group ($p < 0.05$).

At the fourth and the fifth day examination no significant differences were found in the diarrhoea remission rate between the two groups ($p > 0.05$). No kind of statistical test was analysed on the 6th and 7th days.

Table2.

Distribution of stool texture in the synbiotic group and control group during hospitalisation in Gastroenterology division Department of Child Health Dr. Soetomo Hospital Surabaya, February-March 2004 (N=50)

Day	Synbiotic group			Control Group			<i>p</i>
	Liquid	Sticky	Solid and formed	Liquid	Sticky	Solid and formed	
1	25	-	-	25	-	-	0.702**
2	7	4	14	19	5	1	0.000*
3	-	-	25	5	3	17	0.002*
4	-	-	25	1	1	23	0.153**
5	-	-	25	-	1	24	1.000 **
6	-	-	-	-	-	-	- ***
7	-	-	-	-	-	-	- ***

*= Significant, ** = non significant; *** = not analysis

Table 2 shows that both groups showed decreasing numbers of patients with a liquid stool every day during the study period. And the improvement of the formed stool become solid at the synbiotic group on the second day in 14 patients (56%), while in the control group it was 1 patient (4%). There was a significant difference in stool changes between the synbiotic group and the control group on the second and third day of the treatment ($p < 0.05$).

Table 3.

Distribution of stool excretion frequency in each day in synbiotic group and control group during hospitalisation in Gastroenterology division Department of Child Health Dr. Soetomo Hospital Surabaya, February-March 2004

Day	Synbiotic Rate			Control rate			t	df	p
	N	Mean	SD	N	Mean	SD			
1	25	7.96	2.951	25	8.8	4.193	-0.819	48	0.417*
2	25	2.12	1.394	25	4.72	2.052	-5.241	43	0.000**
3	25	1.00	0.000	25	2.2	1.756	-3.417	48	0.002**
4	25	1.00	0.000	25	1.16	0.800	-1.000	48	0.327*
5	25	1.00	0.000	25	1.16	0.800	-1.000	48	0.327*
6	-	-	-	-	-	-	-	-	- ***
7	-	-	-	-	-	-	-	-	- ***

t-test with free sample; * =non significant; ** =significant ***= not analysed

In this study the result of t-test with two free samples, significant differences were found in the mean value of stool excretion frequency in acute diarrhoea in each day between synbiotic group and the control group ($p < 0.05$) at the second and third day.

Diarrhoea time remission

Mean values of remission time in the hospital diarrhoea patient from the synbiotic group and the control group are 37.36 hours and 72.4 hours (table 4). Significant differences were in the term of remission time in the hospital between synbiotic group and the control group ($p < 0.05$).

Table 4.

Mean value of remission time (hour) acute diarrhoeal patient synbiotic group and control in Gastroenterology division Department of Child Health Dr. Soetomo Hospital Surabaya, February-March 2004

Treatment	N	Mean (hours)	SD
Synbiotic group	25	37.36	12.038
Control Group	25	72.40	16.266
Total sum	50	54.88	22.67

t-test with two free samples; $t = - 8.658$; $df = 48$; $p = 0,000$ (significant)

Table 5.

Distribution of remission time related to main enteropathogen in the synbiotic group and the control group in Gastroenterology division Department of Child Health Dr. Soetomo Hospital Surabaya, February-March 2004

Enteropathogen	Synbiotic group			Control group			p
	N	Mean (hour)	SD	N	Mean (hour)	SD	
Rotavirus	9	41.33	12.649	11	74.91	20.062	0.000*
EPEC	11	37.64	11.129	6	77.67	25.812	0.011*

t-test with two free samples; * = significant

As we see from the main enteropatogen the result of stool culture in this research also found significant differences between both groups ($p < 0.05$) (table 5).

Table6.

Distribution of remission time related to malabsorption in synbiotic group and the control group in Gastroenterology division Department of Child Health Dr. Soetomo Hospital Surabaya, February-March 2004

Positive malabsorbtion stool	Synbiotic Group			Control group			p
	N	Mean (hour)	SD	N	Mean (hour)	SD	
<i>Clini test</i>	3	34.67	13.614	6	86	19.839	0.005*
<i>Floating test</i>	21	36.86	12.289	19	70.86	12.517	0.000*

t-test with two free samples; * = significant

Table 6 shows the result of positive malabsorption tests in both of the groups. The synbiotic group shows faster remission time, significant differences were found between the two groups ($p < 0.05$).

DISCUSSION

Based on the homogeneity analysis of characteristics of data, confounding variables and experimental design (randomised double blind placebo control clinical trial) it can be concluded that the study result is caused by the administration of synbiotic.

Stool form analysis using Chi square test showed that the improvement of stool texture in the synbiotic group was significantly faster than the control group. It means that synbiotics could accelerate changes of stool form that support the clinical improvement.

The faster improvement in stool texture and decrease in stool frequency in the synbiotic group lead to a reduction in stool output.

Assessment of remission rate of acute diarrhoea in this study was measured by changes of stool form and frequency of stool output that were observed every day.

The clinical benefit of administering synbiotics in this study was based on the analysis of diarrhoeal remission time that can be related to the role of synbiotics on the gastrointestinal function. Remission time analysis, whether it was related to pathogen EPEC or rotavirus showed there was a significant difference in the synbiotic group compared to the control group.

The benefit of using probiotic in diarrhoeal disease is based on the capability of probiotic bacteria producing lactase that may help lactose digestion in diarrhoeal patients who quite common suffering from lactase deficiency (Fuller, 1991).

This study showed that the synbiotics improved diarrhoea in lactase deficient patients in the synbiotic group compared to the lactase deficient patients in the control group. Lactase deficiency in this study is expressed by positive lactose malabsorption test (table 6).

Many strains of probiotic bacteria that produce lactase also produce a substance like bacteriocin that having antibiotic properties for eradicating pathogens that cause diarrhoea (Fuller, 1991).

Metabolic results of probiotic bacteria are lactic acid, acetic acid, and propionic acid that classified as short chain fatty acids are capable of creating acid milieu and this milieu is unfavourable for growing pathogenic bacteria. Short chain fatty acids are easily absorbed by enterocytes that support energy for enterocyte metabolism and subsequently increase enterocyte survival (Bengmark, 1998; MacFarlane, 1999).

The occurrence of fat malabsorption in this study was quite high, the patients with fat malabsorption who received synbiotic clinically improved rapidly. It is still difficult to explain whether probiotic bacteria produced lipase or because of probiotic save and promote enterocytes survival that capable to produce intestinal lipase. More research is still needed to elucidate this phenomenon.

Small intestinal mucosal injury in acute diarrhoea leads to transient fat malabsorption. Rapid recovery of epithelial cells will occur if mucosal injury was discontinued by administration of probiotic which contains the bacteria capable of suppressing the growth of pathogenic bacteria. In the group of fat malabsorption, synbiotics significantly shortened the course of diarrhoea, compared to the control group in which fat malabsorption was not treated and improved naturally. In this study improvement of lactose intolerance and fat malabsorption contributed to the improvement of the diarrhoea.

Other researchers reported that the administration of Lactobacillus GG probiotic to acute diarrhoeal patients in the form of mixing with milk or probiotic powder improved diarrhoea rapidly (Isolauri, 1991), Bifidobacteria dissolved in milk formula also gave good results (Rochim, 2001). Combining Lactobacillus GG with oralyte promote shortening diarrhoeal episodes. Metaanalysis studies of probiotics for acute diarrhoea have provided a lot of benefits (Van Niel, 2002).

Related to enteropathogen found in this study, synbiotics may regulate bacterial ecosystems in the gastrointestinal tract as expressed by reducing the number of patients with positive stool culture for EPEC from 40% at the beginning to 8% at the end of study and from 36% positive for rotavirus to 12%.

Significant reductions of positive stool cultures influenced by synbiotic may be explained through the mode of action of these bacteria based on the in vitro and in vivo studies. Firstly probiotics will compete with other pathogenic bacteria in colonising mucosal epithelial cells, the presence of probiotics that govern enterocytes promote absorption of adhering pathogenic bacteria to mucosa (Camp, 1985; Bernet, 1994; Bengmark, 1998; McFarlane, 1999).

As the growth of pathogenic bacteria in the lumen of the intestine is suppressed by probiotics, the direct effect of pathogens or secreted enterotoxins to induce diarrhoea will decrease.

Prebiotics are carbohydrate Fructooligosaccharide that is very important substances to speed up the selective growth of probiotic bacteria (Robertfroid, 2000).

Synbiotics improve the life defence of probiotics because the specific substrate has been provided for fermentation so the body will get more benefit from this kind of combination (Collin, 1999)

Other than through the competition mechanism, probiotic bacteria also have the ability to produce an antimicrobial substance; which consists of lactic acid, hydrogen peroxide, bacteriocyn, nicyn, organic acid, microsyn, reuteryn, volatile fatty acid and hydrogen ion, also proteolytic ability to degrade protein in order to prevent the virus (Millar, 1993; Alm, 1999).

Another important mechanism of the action of probiotics is through the induction of mucosal immune response. Many reports have stated that probiotics are capable of affecting mucosal and systemic immune responses (Alm, 1999; McFarlane, 1999; Mc Cracken, 1999 dan Cebra, 1999). Most of immunocompetence cells, mucosal epithelial cells, T and B cells can be influenced by probiotic bacteria or its products including, lipopolysaccharide (LPS), peptidoglycan, lipoteichoic acid (LTA), so that immune responses will be evoked.

Bifidobacteria have an affinity to bind enterocytes cell membranes and may act as carriers for antigens to generate mucosal immune response. Intestinal microbiota like Lactobacilli, Enterococci, slow lactose fermenting coliform as commensal flora have an ability to generate mucosal immune responses as expressed by the appearance of Ig A producing cells in lamina propria whether to germ free mice or normal mice.

Probiotics may induce the changes of immunocompetent cells, CD4 T cells in Peyer's patches from naïve CD45RB^{high} to activated CD45RB^{low}, CD8 T cells, and NK cells. Even probiotics as commensal bacteria are capable of generating local humoral and cellular mucosal immune response.

Even though probiotic bacteria have a capability to induce mucosal immune response, it takes around a week to get the appropriate function as immune exclusion on the surface of intestinal mucosa represented by high level of SIgA. Based on this phenomenon it is unlikely that immune response is involved in shortening the course of diarrhoea.

Prolonged administration of synbiotics will promote continuous stimulation of mucosal immune response that may play an important role in the protection and prevention of gastrointestinal infection.

It is expected that the source of infection can be minimized by administering probiotics during an episode of diarrhoea because probiotics have a capability to regulate gastrointestinal microecosystem by reducing pathogenic bacteria.

CONCLUSION

From this study it can be concluded that the synbiotic tested will shorten the course of diarrhoea through its influence on the rapid improvement of stool formation and reduce stool frequency.

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